ABSTRACT

Methods using gel electrophoresis and mass spectrometry for the rapid, quantitative analysis of proteins or protein function in mixtures of proteins derived from two or more samples in one unit operation are disclosed. In one embodiment the method includes (a) preparing an extract of proteins from each of at least two different samples; (b) providing a set of substantially chemically identical and differentially isotopically labeled protein reagents, one for each sample; (c) reacting each protein sample of step (a) with a different reagent from the set of step (b) to provide isotopically labeled proteins; (d) mixing each of said isotopically labeled proteins to form a single mixture of different isotopically labeled proteins; (e) electrophoresing the mixture of step (d) by an electrophoresing method capable of separating proteins within said mixture; and (f) detecting the difference in the expression levels of the proteins in the two samples by mass spectrometry based on individual peptides derived from chemical or enzymatic digestion. The analytical method can be used for qualitative and particularly for quantitative analysis of global protein expression profiles in cells and tissues, i.e. the quantitative analysis of proteomes.